

Extraction and Efficiency of Chitosan from Shrimp Exoskeletons as Coagulant for Lentic Water Bodies

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Abstract

Artificial lentic water bodies such as lakes created by dams and reservoirs are important sources of supply for drinking water in urban and rural areas of developing countries. Nonetheless, typical low turbidity and high color concentrations of this kind of water bodies make it difficult to use synthetic coagulants such as aluminum and iron salts. Chitosan can be a potential coagulant.

A 3.2×10^6 g mol⁻¹ molecular weight chitosan was obtained from shrimp exoskeletons, with a 72.9% deacetylation degree indicating process effectiveness. Highest coagulant activity of chitosan (89%) was obtained when applied in a 15 mg / L dose of raw and low turbidity water (67 NTU). No statistically significant changes were present in pH, alkalinity and color of water after treatment. Chitosan proved to be an excellent alternative for coagulation-flocculation processes of lentic waters bodies.

Keywords: lentic water bodies, chitosan, coagulant activity, pH, alkalinity, color

INTRODUCTION

Lentic water bodies are defined as those with surface temporarily or permanently covered by water, except those occasionally inundated landscape areas [1]. They are a valuable source of water supply for developing countries, particularly, regions without permanent surface water currents such as rivers or streams. Nonetheless, nutrient inputs due to surface runoff and prolonged water retention times in these aquatic systems promote algae growth, and consequently, eutrophication phenomena [2]. Recently, alternative systems have been implemented for eutrophic water treatment, in which high concentrations of organic matter prevent use of conventional water treatment methods [3], including use of natural polymeric coagulants such as chitosan [4].

Coagulation/flocculation is a common technique widely used

in many industrial applications promoting solid/liquid separation processes, due to profitability and straightforward operation [5-8]. Addition of a polymeric flocculant allows destabilization of colloidal particles in suspension due to neutralization of negative charges [5], facilitating formation of precipitable flocs and favoring a considerable increase in sedimentation rate [7].

Typically, for this unitary process, inorganic salts of polyvalent metals have been widely used for decades due to low cost [9]. However, large doses of these synthetic coagulants are required for efficient flocculation, leading to production of large volumes of metal hydroxide sludge. Inadequate end disposal of these sludge represents a serious environmental problem [10]. Synthetic coagulants have a high sensitivity to pH, low efficiency in removing very fine particles and are only applicable to a few dispersed systems; being this another drawback for their use [11, 12]. On the other hand, an increase in the concentration of coagulant metal in treated water can have serious problems in human health [13, 14].

Organic polymeric coagulants, unlike synthetic ones, are highly efficient at small doses (e.g., few milligrams per liter) and produce a much smaller sludge volume without alkalinity consumption. Furthermore, flocs formed during process are bigger and stronger, thus, with excellent sedimentation characteristics [15, 16].

A lot of research is focused on replacement of conventional synthetic coagulants with biological-based coagulants due to the advantages over them [17]. Recently, use of chitosan and its derivatives as coagulants is booming, due to wide availability, environment compatibility and excellent structural characteristics [8]. Additionally, it has been reported as a non-toxic and safe compound for human health [18].

Chitosan is a poly-β-(1-4)-D-glucosamine derived from partial chitin deacetylation, a polysaccharide found in many insects, marine invertebrates exoskeletons, fungi and some algae [19, 20]. When deacetylation degree reaches 50% approximately

chitin is generally soluble in aqueous acidic environment and is called chitosan [21]. Nevertheless, its solubility depends on deacetylation grade and molecular weight [22].

Deacetylation Degree (DD) and molecular weight (MW) are two paramount structural factors for chitosan. When chitosan is used as a flocculant, DD is related to charge density and strongly influences, like MW, efficiency of charge neutralization and morphology and polymer chain conformation in the aqueous environment. Likewise, all these structural factors interact with several environmental factors (dose, pH, ionic strength and temperature) and influence the performance of flocculants [8].

Flocculation mechanisms occurring due to activities of various polymeric flocculants, including chitosan and derivatives, can be categorized as: complete charge neutralization, partial charge neutralization, bridging and sweeping [23, 24].

This study aims at extracting chitosan from shrimp exoskeletons, evaluating its coagulant activity in the treatment of raw water from a lentic water body and evaluating its influence on pH, alkalinity and color after treatment.

MATERIALS AND METHODS

Raw Water Samples

Samples of raw water were taken from an artificial reservoir located in the rural area of Sincelejo city, Colombia, at 9° 16'12" N - 75° 20'30" W coordinates. Simple samplings were carried out between March and October 2016, at the end of the dry season and start of the rainy season.

Raw Material for Chitosan

Shrimp exoskeletons from *Plaeomon serratus* species and collected from a seafood restaurant in Monteria city, Colombia were used. Exoskeletons were dried at room temperature for 1 day, treated then in boiling water with constant agitation for 1 hour and finally washed with clean water. Residues were dried at room temperature and sieved in a # 20 mesh (841µm) to eliminate small foreign matter [25]. Later, grinding was carried out through a manual grinding machine, sieved in a # 60 mesh (250µm) where a powder of a fine particle size was obtained and stored in a dry place at room temperature until extraction [26]. All chemical products (H₂SO₄, NaOH, NH₄OH & C₂H₄O₂) used in the study were of analytical quality and were purchased from Merkal KGaA (Darmstadt, Germany).

Demineralization

The dry powder was dissolved in a 1% (v/v) acetic acid solution in a 1/20 (w/v) ratio, constantly stirring for 30 minutes and then every 20 minutes until completing 4 hours. After that, precipitate was isolated, washed with pure water and sieved in a # 200 mesh (74µm) until reaching a neutral pH [25].

Deproteinization

Once demineralized, powder deproteinization was carried out with alkaline solution of sodium hydroxide (2N) in a 1/20 (w/v) ratio for 2 hours and constantly stirred at room temperature. At the end, sample was filtered, washed, dried and stored at room temperature [27].

Chitosan Production

The demineralized and deproteinized powder was deacetylated using a domestic microwave. 10 grams of powder and 100 ml of 50% strong alkaline solution (w/v) of sodium hydroxide were added in a 200 ml beaker, and manually stirred with a glass rod for 10 minutes. The solution was placed on the turntable's center of a microwave oven (Samsung model microwave oven, AMW831K, 800 Watts, made in Korea) and irradiated for 20 minutes at 800 W. Mixture and mixture residue were sieved again in a # 200 mesh (74 µm) and washed with potable water to a neutral pH, then rinsed with deionized distilled water and dried in a hot-air oven at 40°C until reaching a constant weight. Finally, stored until use in the residual water treatability tests [24].

For extracting the liquid coagulant, chitosan powder was dissolved in a 1% acetic acid solution with a constant stirring of 150 rpm for 12 hours, to get a stock solution of 3 g L⁻¹.

Viscosity Average Molar Weight

To determine the polymer molecular weight, a high precision AMVn Anton Paar GMBH microviscometer (Quebec, Canada) at 20°C was used. Chitosan solutions were prepared using a 0.1M acetic acid and 0.2M sodium chloride solution as solvent, according to the method proposed by [28] and [29]. The polymer initial concentration was 4.0x10⁻³ g mL⁻¹. Once working conditions were established, polymer solution fall time in the capillary was determined. The relationship between the intrinsic viscosity and the polymer molecular weight was determined by the Mark-Houwink equation:

$$[\eta] = K (M_{\eta})^{\alpha} \quad \text{Equation 1}$$

Parameters K and α are called Mark-Houwink constants which depend on polymer nature, solvent and temperature [28]. K and α values used were 1.81x10⁻³ mL g⁻¹ and 0.93, respectively [30-32]. This procedure was repeated for three solutions additional with concentrations of 1x10⁻³, 2x10⁻⁴, 6x10⁻⁴ g mL⁻¹ each. Density measurements of the different solutions were determined by using the pycnometric method.

Determination of Deacetylation Degree (DD%)

FTIR spectra were recorded on a device with a Shimadzu IRTracer 100 spectrometer with type IIA ATR (Attenuated Total Reflectance) with diamond crystal, at 25°C, with a wave number range between 4000-550 cm⁻¹ and a 4 cm⁻¹ resolution. All spectra were recorded with 64 scans per test, in transmittance mode. Degree of acetylation and deacetylation

(DD%) of chitosan was carried out according to the procedure proposed by [33] and [34].

Jar Tests

A jar test, on an E & Q flocculator model F6-330-T, was performed to determine optimal required coagulant dose, capable of removing as much turbidity as possible from wastewater. Chitosan Coagulant extracts were applied with doses of 5, 10 and 15 mg L⁻¹ to each raw water sample with initial turbidity of 67, 103 and 164 NTU, according to the proposed experimental design. Rapid mixing process was maintained at 200 rpm for 1 minute, while it was 40 rpm for 20 minutes for the slow mixture. Samples were sedimented for 20 minutes and residual turbidity was measured then with a HACH 2001P Turbidimeter according to the standardized methods of the American Public Health Association [35]. In all jar tests, a blank was used to verify chitosan activity in the turbidity removal, calculated with the following equation [36-38]:

% Coagulant Activity =

$$\frac{\text{Residual Turbidity}_{\text{blank}} - \text{Residual turbidity}_{\text{sample}}}{\text{Residual Turbidity}_{\text{blank}}} * 100$$

Equation 2

Experimental Design

A 3² factorial design was applied for the experimental design. Two factors were evaluated in three levels each and a replica of each test was made, yielding a total of 18 tests for the coagulant plus 3 blanks. Factors analyzed were initial turbidity of raw water and applied doses of coagulant based on chitosan. This design was made to determine each factor effect, i.e., change in response (for this case, chitosan coagulant activity), produced by a change in the factor level. Interaction between factors and effect on the system response in pH, alkalinity and true color in samples was also analyzed. An analysis of variance (ANOVA) was the statistical tool implemented for data analysis, by using the R program and the Statgraphics Centurion XVI (Version 16.0.07). For all statistical analyzes, significance level was established at p <0.05.

RESULTS AND DISCUSSION

Deacetylation Degree of Chitosan (DD%)

Figure 1 shows FTIR spectra obtained for the tested chitosan samples. Distinctive compound peaks can be distinguished. The band located at 1651 cm⁻¹ corresponds to the peak of Amide I and the band located at 1627 cm⁻¹ corresponds to the C-N stretch vibration of the superimposed C = O of

Amide I.

The band located at 1560 cm⁻¹ is a stretch or deformation band of amide II. In the 1373 cm⁻¹ band, the symmetric deformation corresponding to CH₃ groups was observed; and in the region between 1334 and 1280 cm⁻¹ in the spectrum, the CO-NH deformation of the CH₂ group of Amide III. The band 1064 cm⁻¹ corresponds to the stretching of the C-O-C bridge of the polysaccharide residue, which could be due to glucosamine. The results obtained are concordant with those reported by other authors [38-43].

According to [33] and [34] it is possible to estimate DD% through the relation between reference bands of amide III located at 1320 cm⁻¹ and the band corresponding to the absorption of methyl groups located at 1420 cm⁻¹, according to the following equation:

$$(DD\%) = 100 - (31.92 * \left(\frac{A_{1320}}{A_{1420}}\right) - 12,2) \quad \text{Equation 3}$$

A1320 represents absorbance value at 1320 cm⁻¹ and A1420 corresponds to absorbance at 1420 cm⁻¹. The result obtained from DD% for the chitosan sample was 72.9%. DD% values equal to 64% have been reported for chitosan extracted from shrimp exoskeletons, suitable for environmental applications [44]. Other authors reported values of DD% between 70% and 80% in chitosan samples extracted from diverse sources (fungi, crustaceans and shrimp) [43] and commercial chitosan [45]. Thus, the chitosan extracting process used in this research study was efficient and the resulting product could be used for diverse applications such as obtaining hydrogels, coatings, films, capsules, coagulating agent and others.

Chitosan Molecular Weight

3.2x10⁶ g mol⁻¹ was the molecular weight obtained for chitosan from shrimp exoskeleton *Plaeomon serratus*. This value is like values reported by other authors [46].

According to [28] the molecular weight is related to polymer deacetylation degree. The chitosan in this study showed a 72.9% (DD%), like the values obtained by these authors, indicating that the process used in the study to extract it was effective.

Physicochemical characteristics of Raw Water Samples

Table 1 shows main physicochemical characteristics of raw water samples taken from the reservoir for treatability tests.

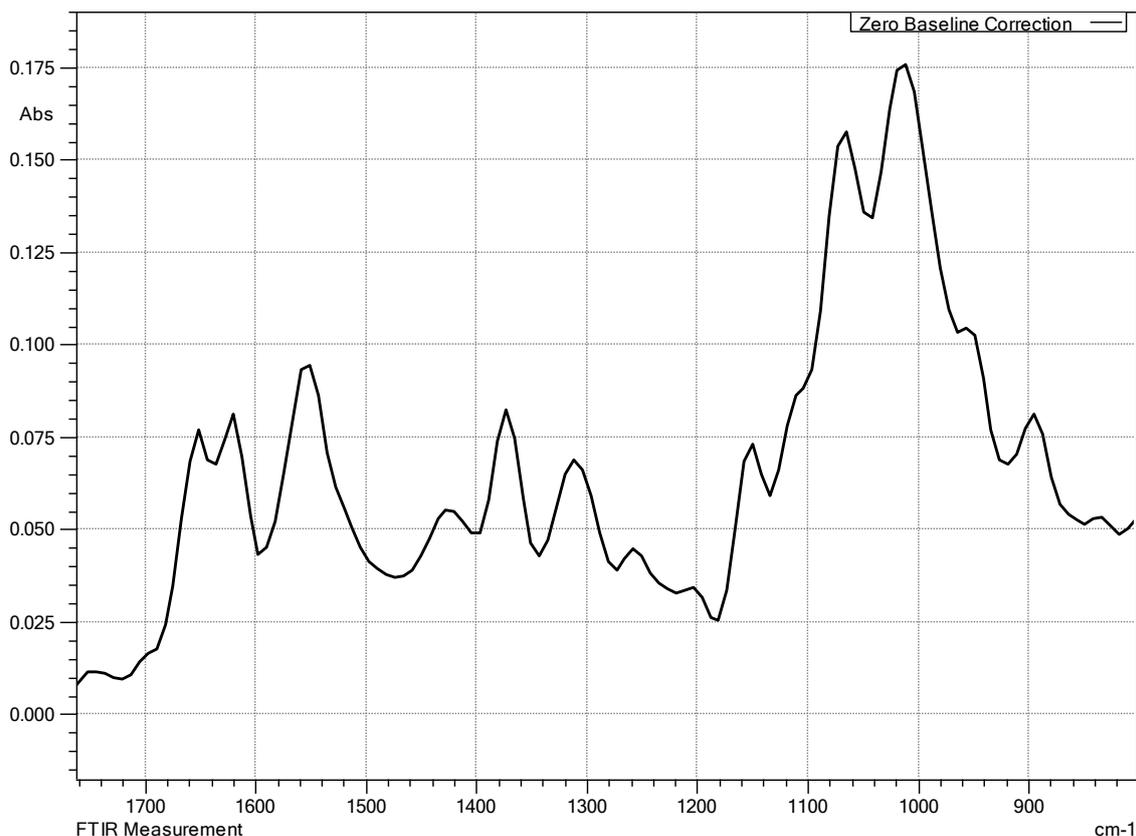


Figure 1: FTIR Spectra for Chitosan

Table 1. Physicochemical characteristics of raw water samples

Parameters	Sample 1	Sample 2	Sample 3
Initial Turbidity (NTU)	67.00	103.00	164.00
pH	7.79	8.09	8.04
Real Color (CU)	9.67	16.33	16.80
Alkalinity (mgCaCO ₃ /L)	40.00	34.00	36.00

Turbidity of the samples taken are typical of water bodies of low turbidity, even in rainy season (sample 3), such as the reservoir selected for this research. pH showed a tendency toward alkalinity, without leaving the established range according to the Colombian regulation for drinking water (6.5 to 9.0 pH units). Color of samples with turbidity of 103 NTU and 164 NTU, is higher than the maximum value established in the Colombian regulation (15 CU), which could indicate a possible relationship between the two variables, i.e., the greater turbidity in the reservoir, the more likely to find a higher color. Alkalinity showed lower values than the norm (200 mg L⁻¹) [47], however, alkalinity is important in water treatment because it reacts with coagulants of iron and aluminum salts in

flocculation processes. Consequently, waters with alkalinity of less than 15 mgCaCO₃ L⁻¹ will hardly form precipitable flocs with these coagulating salts [48].

Chitosan Coagulant Activity

The coagulating activity of chitosan extract on raw water samples taken from the reservoir is shown in Figure 2.

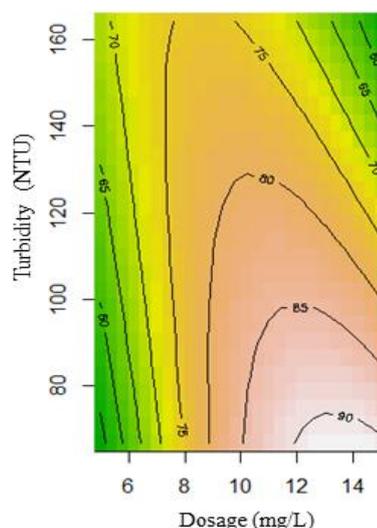


Figure 2: Diagram of Chitosan coagulant activity

According to the extracted coagulation diagram, the greatest coagulant activity of chitosan occurs when water turbidity is less than 100 NTU (between 85% and 90%), with doses between 10 mg L⁻¹ and 15 mg L⁻¹. As turbidity of raw water increases, coagulant activity decreases, however, best efficiency occurs with doses between 5 mg L⁻¹ and 10 mg L⁻¹ (between 55% and 84%, respectively). Synthetic coagulants have optimal efficiencies in raw water with high turbidity, high alkalinity and in a certain pH range [49]. Therefore, they could not be used efficiently in the reservoir studied. Based on results

obtained in this study, the chitosan extract coagulant is ideal for this type of water, because its action is better when turbidity is low.

Chitosan Influence on pH, Alkalinity and Color in Treated Water

Figure 3 shows results of pH, alkalinity and color after applying doses of chitosan coagulant on three samples of raw water from the reservoir.

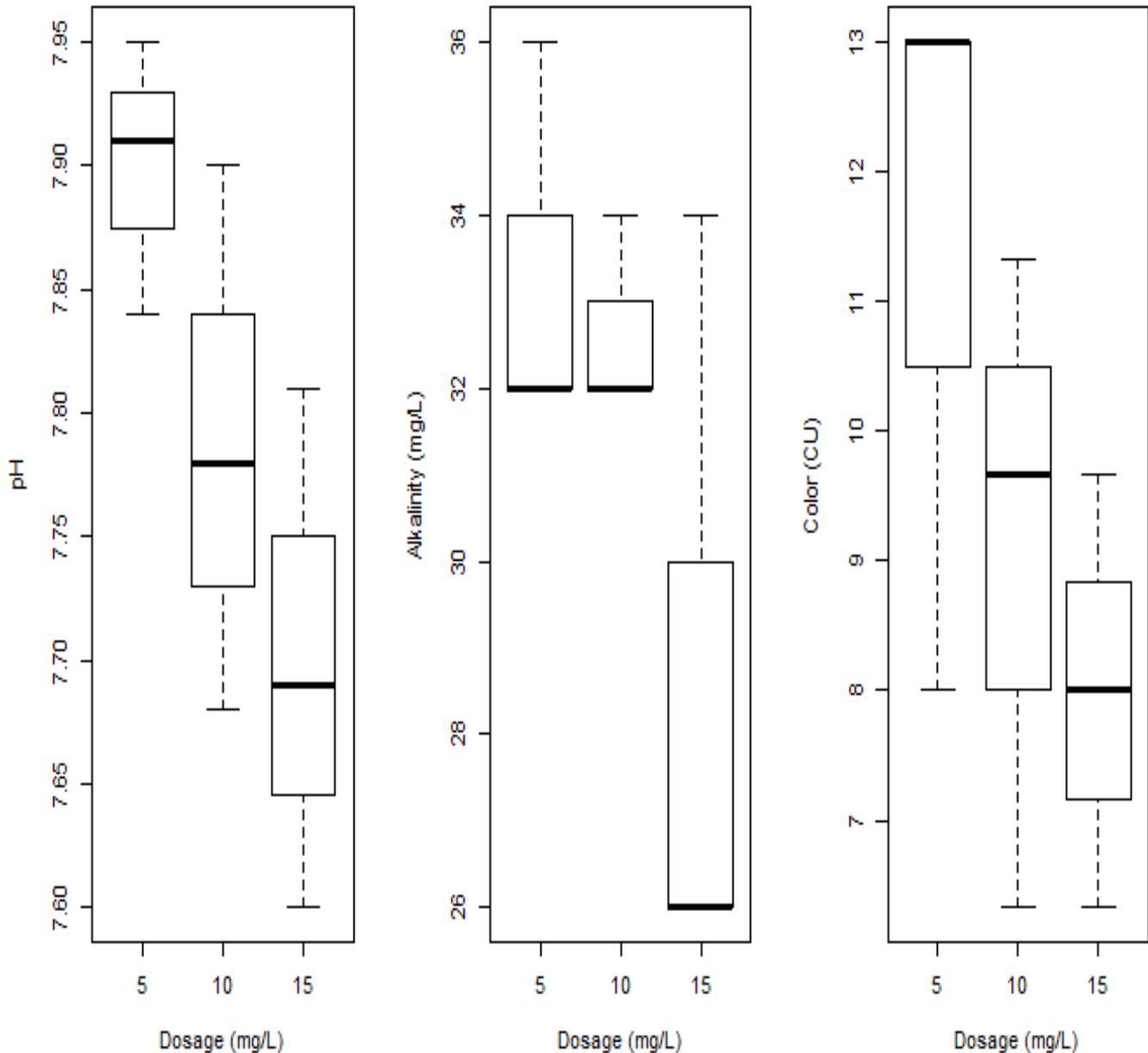


Figure 3: Behavior of pH, Alkalinity and Color

It was observed that pH concentration, alkalinity and color tend to decrease depending on the increase of coagulant doses, this may be due to the coagulant acid nature, because the extract is prepared in an acid environment (1% acetic acid). For evaluating the significant effect of coagulant doses on raw water samples, an analysis of variance (ANOVA) was used. For all ANOVAS, assumptions of independence, normality and homogeneity of variances were verified, based on the tests of Durbin Watson, Shapiro-Wilk and Levene respectively. P values are shown in Table 2.

Table 2. ANOVA P Values

Parameters	P Values
pH	0.84
Alkalinity (mgCaCO ₃ /L)	0.14
Real Color (CU)	0.59

"P" values for pH, alkalinity and real color, after applying coagulant extract of chitosan to raw water samples, were greater than 0.05, i.e. there is no statistically significant difference between the means of these parameters, with a 95% confidence level. In other words, chitosan as a coagulant does not significantly alter physicochemical characteristics of water after treatment. Unlike inorganic salts, which significantly alter pH and alkalinity characteristics of water after coagulation process [49], the coagulant of chitosan extract does not significantly influence these parameters; making it a large advantage over synthetic coagulants. This is interesting from the point of its direct application in a potabilization plant, since water pH must not be corrected at the input of the process [50, 51].

CONCLUSIONS

Through physical and chemical process applied to shrimp exoskeletons from the *Plaeomon serratus* species for extracting chitosan, proposed in this study, it was possible to obtain chitosan of decent quality, with excellent molecular weight characteristics and deacetylation degree. Chitosan turned out to be ideal for coagulation-flocculation treatments of lentic water bodies, due to its high coagulant activity in waters of low turbidity, low alkalinity and high color.

pH, alkalinity and color of samples treated with natural coagulant based on chitosan, did not have significant changes after doses application. The above is an advantage over synthetic coagulants, which alter original pH and alkalinity conditions of raw water.

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